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# Ear rot, aflatoxin accumulation, and fungal biomass in maize after inoculation with *Aspergillus flavus*☆

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ABSTRACT

Aflatoxin, a toxin produced by the fungus *Aspergillus flavus* Link: Fries, occurs naturally in maize (*Zea mays* L.). Aflatoxin is a potent human carcinogen and is also toxic to livestock, pets, and wildlife. When contaminated with aflatoxin, the value of maize grain is markedly reduced. This investigation was conducted to compare ear rot, aflatoxin accumulation, and fungal biomass in maize single crosses with varying degrees of resistance to aflatoxin accumulation and to determine the relative importance of general combining ability (GCA) and specific combining ability (SCA) in the inheritance of resistance to ear rot, aflatoxin accumulation, and fungal biomass. Eight germplasm lines with different levels of resistance to aflatoxin accumulation were used as parents of a diallel cross. The cross was evaluated for visible ear rot, aflatoxin accumulation, and *A. flavus* infection in the grain. *A. flavus* infection was determined by quantitative real-time polymerase chain reaction (qRT-PCR) assays. Both GCA and SCA were significant sources of variation in the inheritance of the three traits although GCA accounted for a greater portion of the variation among single crosses. The interactions of GCA and SCA with years were highly significant for aflatoxin accumulation, but not significant for *A. flavus* infection. Estimates of GCA effects were highly significant for both reduced *A. flavus* infection and reduced aflatoxin accumulation for Mp313E, Mp715, and Mp717. Conversely, GCA effects associated with GA209 were significant for reduced levels of *A. flavus* infection and ear rot, but high levels of aflatoxin accumulation. Mp313E, Mp715, and Mp717 should be useful in breeding programs targeting both reduced levels of fungal infection and aflatoxin accumulation.

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## 1. Introduction

Aflatoxin is produced by the fungus *Aspergillus flavus* Link: Fries and occurs naturally in maize (*Zea mays* L.). Aflatoxin is the most potent carcinogen found in nature, and consumption of aflatoxin-contaminated foods is a major cause of hepatocellular carcinoma in humans (Castegnaro and McGregor, 1998; Wild and Hall, 2000). It is also toxic to livestock, pets, and wildlife (Gourama and Bullerman, 1995; Leung et al., 2006).

The presence of aflatoxin in grain substantially reduces its value. Drought and high temperatures are frequently associated with high levels of aflatoxin accumulation. In the United States, aflatoxin contamination of pre-harvest maize is a sporadic problem in the Midwest, but a chronic problem in the Southeast (Payne, 1992; Widstrom, 1996). The U.S. Food and Drug Administration has set a tolerance of 20 ng/g for aflatoxin B1, the most common form of aflatoxin found in maize grain. Grain with higher concentrations is restricted from interstate commerce (Gourama and Bullerman, 1995).

Host-plant resistance to *A. flavus* infection and subsequent aflatoxin accumulation is generally considered a highly desirable means of reducing losses to aflatoxin. Working in Mississippi, Scott and Zummo (1988) developed techniques for screening maize germplasm for resistance to *A. flavus* kernel infection. They developed and released Mp313E and Mp420, the first maize germplasm lines released as sources of resistance to *A. flavus* infection and subsequent aflatoxin accumulation (Scott and Zummo, 1990, 1992). Two additional germplasm lines developed in Mississippi, Mp715 and Mp717, have also been released (Williams and Windham, 2001, 2006). These lines were selected for low levels of aflatoxin accumulation. In field tests conducted in Mississippi in 2007 and 2008, Mp313E, Mp715, and Mp717 exhibited low levels of both ear rot and aflatoxin accumulation after inoculation with *A. flavus* (Henry et al., 2009).

Published reports on the association between *A. flavus* infection and aflatoxin accumulation are contradictory. From the results of field tests conducted in Illinois, Campbell and White (1995) concluded that Aspergillus ear rot and aflatoxin accumulation were

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**Table 1**
Analysis of variance for ear rot, aflatoxin concentration, and *A. flavus* biomass in maize grain harvested from a diallel set of crosses inoculated in field trials grown at Mississippi State in 2008 and 2009.

| Source | df | Mean squares | | |
|---|---|---|---|---|
| | | Ear rot[a] | Aflatoxin[b] | *A. flavus* biomass[c] |
| Years | 1 | 0.3 | 21.37** | 600.10** |
| Reps (years) | 6 | 100.6 | 1.31 | 1.02 |
| Hybrids | 27 | 288.7* | 16.38** | 9.83** |
| GCA[d] | 7 | 639.5** | 53.74** | 21.81** |
| SCA | 20 | 164.6** | 3.30** | 5.64** |
| Hybrids × years | 27 | 118.2* | 2.57** | 2.26 |
| GCA × years | 7 | 146.2* | 4.93** | 1.62 |
| SCA × years | 20 | 108.4 | 1.75** | 2.49 |
| Error | 161 | 66.7 | 0.83 | 2.27 |
| $R^2$ | | 0.51 | 0.80 | 0.72 |

[a] Ear rot was visually rated at harvest as a percentage of rotted kernels on 10 ears per plot.
[b] Aflatoxin concentration was determined in 50-g grain samples and values transformed [ln($y$ + 1)] before statistical analysis.
[c] *A. flavus* biomass was calculated as the actual ratio of *A. flavus* to maize biomass based on extracted genomic DNA from inoculated ears.
[d] GCA, general combining ability; SCA, specific combining ability.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

not highly correlated. They found, however, that inbred line LB31 exhibited consistently high levels of resistance to both ear rot and aflatoxin accumulation. In a subsequent investigation, the correlation between Aspergillus ear rot and aflatoxin accumulation was determined to be significant and positive (Walker and White, 2001).

To study the relationship between fungal biomass and aflatoxin accumulation in maize grain, Mideros et al. (2009) developed and validated two quantitative real-time polymerase chain reaction (qRT-PCR) assays to estimate fungal biomass in maize tissues. Fungal biomass and aflatoxin concentration were assayed in grain samples from a set of hybrids and a set of inbred lines grown in Mississippi and inoculated with *A. flavus*. For both hybrids and inbred lines, fungal biomass and aflatoxin concentration were highly correlated.

This investigation was conducted to compare ear rot, aflatoxin accumulation, and fungal biomass in crosses among eight maize germplasm lines known to have varying degrees of resistance to aflatoxin accumulation. The second objective was to ascertain the importance of general combining ability (GCA) and specific combining ability (SCA) in the inheritance of resistance to ear rot, aflatoxin accumulation, and fungal biomass.

## 2. Materials and methods

A diallel cross was produced from eight maize inbred lines: Mp313E, Mp715, Mp717, NC408, CI66, GA209, SC212m, and T173. Mp313E, Mp715, and Mp717 were developed in Mississippi and released as sources of resistance to *A. flavus* infection and aflatoxin accumulation (Scott and Zummo, 1990; Williams and Windham, 2001, 2006). Mp 313E has white kernels, and Mp715 and Mp717 have yellow kernels. These are late-maturing lines that should be useful in both temperate and sub-tropical breeding programs. CI66, GA209, SC212m, and T173 are highly susceptible to aflatoxin accumulation, and NC408 has exhibited an intermediate level of resistance (Williams, 2006). These lines mature earlier than Mp313E, Mp715, and Mp717.

The 28 $F_1$ hybrids produced by making all possible crosses among the lines were planted on 24 April 2008 and 28 April 2009 at the R.R. Foil Plant Science Research Center, Mississippi State University, Starkville, Mississippi. Hybrids were planted in single-row plots 4m long and spaced 0.97 m apart in a randomized complete block design with four replications. Plots were thinned to 20 plants after seedlings emerged. Standard production practices were followed. Supplemental irrigation was applied as needed.

The primary ear of each plant in a plot was inoculated with *A. flavus* isolate NRRL 3357, which is known to produce aflatoxin, using a tree-marking gun fitted with a 14-gauge needle. Inoculations were made 7 d after silks had emerged from 50% of the plants in a plot. Because the time of silk emergence varied among hybrids, all plots were not inoculated on the same day. A 3.4-ml suspension containing $3 \times 10^8$ *A. flavus* conidia was injected underneath the husks into the side of the ear (Zummo and Scott, 1989). Inoculum was prepared as described by Windham and Williams (2002).

Ten ears were harvested from each plot approximately 60 d after inoculation. Ears were dried for 7 d at 38 °C. Ears from each plot were then examined, and the percentage of rotted kernels was visually estimated for each ear. A mean of the 10 ears from each plot was then determined. The ears from each plot were bulked and shelled using an ALMACO maize ear sheller (Allan Machine Company, Nevada, Iowa, USA). The grain was thoroughly mixed and ground using a Romer mill (Union, Missouri, USA). The concentration of aflatoxin in a 50-g subsample of ground grain was determined by the VICAM Aflatest (Watertown, Massachusetts, USA). This procedure detects aflatoxin at levels as low as 1 ng/g.

Fungal biomass in grain samples was determined by the following procedures. Genomic DNA was extracted from *A. flavus* tissue and *A. flavus* infected and non-infected maize tissue. Species specific primers, Af2 (forward primer: 5′-ATCATTACCGAGTGTAGGGTTCCT-3′; reverse primer: 5′-GCCGAAGCAACTAAGGTACAGTAAA-3′; amplicon 73 bp) designed in the internal transcribed spacer 1 (ITS1) sequence and Zmt3 (forward primer: 5′-TCCTGCTCGACAATGAGGC-3′; reverse primer: 5′-TTGGGCGCTCAATGTCAA-3′; amplicon 63 bp) amplifying maize α-tubulin were used to quantify *A. flavus* and maize, respectively. These primers were designed and reported by Mideros et al. (2009).

Power SYBR green PCR Master Mix (Applied Biosystems, Foster City, California) was used at 1× concentration with 2 μl of sample template (approximately 10 ng/μl) in 10 μl reaction volumes. The PCR conditions were 95 °C for 10 min for initial denaturation, followed by 45 cycles of 95 °C for 5 s, 59 °C for 10 s, and 72 °C for 5 s and a final extension at 72 °C for 5 min. Also, for the purpose of biomass quantification, two standards containing both *A. flavus* DNA and the maize DNA in the ratio 1:1 and 20:1 were included in each run. Both *A. flavus* and maize DNA were quantified in each biological sample and in the standards using the species specific primers Af2 and Zmt3 in separate wells in the same run. Two technical replicates were run for each biological sample and were included in the same run on the same 96-well plate.

**Table 2**
Ear rot, aflatoxin concentration, and *A. flavus* biomass in 28 $F_1$ hybrids constituting a diallel cross grown at Mississippi State in 2008 and 2009.

| Hybrid | Days to midsilk[a] | Ear rot[b] | Aflatoxin[c] | | *A. flavus* biomass[d] |
|---|---|---|---|---|---|
| | | % | ln(ng/g + 1) | ng/g | mg/g |
| NC408 × SC212m | 71 | 18 | 7.17 | 1303 | 7.3 |
| CI66 × T173 | 69 | 21 | 6.76 | 863 | 4.0 |
| GA209 × T173 | 68 | 11 | 6.47 | 645 | 3.1 |
| NC408 × T173 | 67 | 12 | 6.35 | 572 | 2.8 |
| SC212m × T173 | 68 | 23 | 6.30 | 542 | 4.7 |
| CI66 × SC212m | 72 | 25 | 6.18 | 480 | 4.1 |
| CI66 × GA209 | 71 | 10 | 6.15 | 465 | 2.5 |
| Mp715 × T173 | 72 | 31 | 5.98 | 396 | 3.1 |
| Mp313E × T173 | 72 | 19 | 5.92 | 370 | 3.2 |
| GA209 × NC408 | 72 | 9 | 5.67 | 288 | 2.4 |
| Mp313E × SC212m | 74 | 15 | 5.63 | 279 | 3.0 |
| GA209 × SC212m | 74 | 9 | 5.58 | 263 | 3.2 |
| Mp717 × T173 | 72 | 18 | 5.53 | 252 | 2.7 |
| CI66 × NC408 | 72 | 13 | 5.36 | 211 | 2.6 |
| Mp717 × SC212m | 72 | 10 | 5.34 | 181 | 2.3 |
| Mp715 × SC212m | 75 | 12 | 4.81 | 121 | 2.5 |
| GA209 × Mp313E | 74 | 8 | 4.72 | 112 | 2.1 |
| CI66 × Mp717 | 72 | 13 | 4.72 | 111 | 2.1 |
| CI66 × Mp313E | 75 | 14 | 4.68 | 107 | 2.9 |
| CI66 × Mp715 | 74 | 15 | 4.60 | 99 | 2.8 |
| GA209 × Mp715 | 75 | 12 | 4.40 | 80 | 2.1 |
| Mp313E × NC408 | 75 | 14 | 4.21 | 66 | 2.2 |
| GA209 × Mp717 | 70 | 12 | 3.99 | 53 | 2.0 |
| Mp715 × NC408 | 77 | 12 | 3.30 | 26 | 2.1 |
| Mp717 × NC408 | 72 | 11 | 3.23 | 24 | 2.1 |
| Mp313E × Mp717 | 77 | 5 | 2.48 | 11 | 1.9 |
| Mp715 × Mp717 | 76 | 10 | 2.19 | 8 | 2.1 |
| Mp313E × Mp715 | 80 | 5 | 1.40 | 3 | 2.0 |
| LSD (0.05) | 2.1 | 11 | 1.65 | | 1.5 |

[a] Number of days from planting until silks emerged from 50% of the plants in a plot.
[b] Ear rot was visually rated at harvest as a percentage of rotted kernels on 10 ears per plot.
[c] Data were transformed [ln($y$ + 1), where $y$ = aflatoxin concentration] before analysis. Geometric means were calculated by converting logarithmic mean back to original units of measure.
[d] *A. flavus* biomass was calculated as the actual ratio of *A. flavus* to maize biomass based on extracted genomic DNA from inoculated ears.

For the determination of primer efficiencies and for DNA quantification, separate standard curves for each set of primers were generated. For Mzt3, several standards with serial dilutions of maize DNA, 40, 30, 20, 4, 0.8, and 0.16 ng/μl, and for Af2 primers, a series of mixed DNA standards containing varying concentrations of *A. flavus* DNA, 100, 40, 30, 20, 10, 1, 0.1, 0.01, 0.001 ng/μl, in a constant maize DNA concentration of 1 ng/μl were used to construct the standard curve. Additionally, to calculate the actual ratio of *A. flavus* to maize biomass (pathogen to host ratio) in infected maize, several biomass standards were prepared by mixing varying known amounts of *A. flavus* tissue, 40, 8, 1.6, 0.32, 0.064, and 0.0128 mg, to make a total of 100 mg with uninfected maize tissue. DNA extracted from these mixtures were analyzed for both *A. flavus* DNA and maize DNA levels, using the specific primers pairs (Af2 for *A. flavus* and Zmt3 for maize) mentioned above and a standard curve was generated with biomass ratio (pathogen to host) on the *X*-axis and DNA ratio (pathogen to host) on the *Y*-axis. The equation for $R^2$ derived from this standard curve was further used to calculate the amount of fungal contamination (fungal biomass) in the infected maize samples.

Plot means were calculated for ear rot, aflatoxin concentration, and fungal biomass. Aflatoxin concentration was transformed as ln($y$ + 1), where y is the concentration in a sample, to provide a more normally distributed data set before statistical analysis. Data were analyzed using the SAS General Linear Models procedure (SAS Institute, 2003). Variance was partitioned, using DIALLEL-SAS (Zhang and Kang, 1997, 2003) based on Griffing's (1956) Method 4, Model 1, into GCA and SCA components and their interactions with years. To test for significance of GCA and SCA components in the two-year analysis, the interaction between years and the corresponding component was used as the error term for *F*-tests. Estimates of GCA and SCA effects were calculated and their significance determined by *t*-tests. Means for ear rot, aflatoxin concentration, and fungal biomass were compared using Fisher's Protected Least Significant Difference (LSD) at $P = 0.05$ (Steel and Torrie, 1980). Following statistical analysis, the transformed means for aflatoxin concentration were converted to the original units of measurement (geometric means) to facilitate comparisons among genotypes.

## 3. Results and discussion

The analysis of variance indicated highly significant differences between years for aflatoxin accumulation and *A. flavus* biomass in grain, but not for ear rot (Table 1). Differences among hybrids were significant for ear rot, aflatoxin accumulation, and fungal biomass. Although both GCA and SCA were highly significant sources of variation, the portion of the variance attributable to GCA was greater than that attributable to SCA for all traits. For ear rot and fungal biomass, GCA accounted for approximately 60%, and SCA accounted for 40% of the variance among hybrids. For aflatoxin accumulation GCA accounted for 85%; SCA accounted for 15% of the variance. The interaction of GCA × years was significant for ear rot. Both GCA × years and SCA × years were highly significant sources of variation for aflatoxin accumulation. Neither GCA × years nor SCA × years was a significant source of variation for *A. flavus* biomass in maize grain.

The percentage of rotted kernels was lowest for Mp313E × Mp717 and Mp313E × Mp715 (Table 2). These $F_1$ hybrids are crosses between two lines selected for resistance to aflatoxin accumulation; aflatoxin accumulation was lowest in these two hybrids together with Mp715 × Mp717 in the cur-

rent investigation. Ear rot was also low in other crosses such as GA209 × Mp313E, GA 209 × NC408, GA209 × SC212m, and CI66 × GA209. Aflatoxin accumulation was significantly higher in these crosses than in the crosses between two lines selected for resistance. Geometric means for aflatoxin accumulation in GA209 × Mp313E, GA 209 × NC408, GA209 × SC212m, and CI66 × GA209 were 112, 263, 288, and 465 ng/g, respectively, while geometric means for aflatoxin accumulation in Mp313E × Mp717, Mp715 × Mp717, and Mp313E × Mp715 were only 3, 8, and 11 ng/g, respectively.

The level of *A. flavus* biomass in maize grain was highest in the hybrid NC408 × SC212m (7.3 mg/g) and lowest in Mp313E × Mp717 (1.9 mg/g). There were fewer significant differences among $F_1$ hybrids for *A. flavus* biomass than for aflatoxin accumulation. In addition to NC408 × SC212m, only CI66 × T173, SC212m × T173, and CI66 × SC212m had significantly higher fungal biomass than the three hybrids produced by crossing the resistant lines: Mp313E × Mp717, Mp715 × Mp717, and Mp313E × Mp715. Fungal biomass and aflatoxin concentration were highly and positively correlated ($r = 0.90$, $P = 0.0001$). Ear rot and aflatoxin concentration were also correlated ($r = 0.51$, $P = 0.006$) as were ear rot and fungal biomass ($r = 0.55$, $P = 0.002$). The fact that ear rot was evaluated by subjective visual ratings rather than by quantitative procedures like aflatoxin concentration and fungal biomass may have contributed to the lower correlations involving ear rot.

The number of days from planting until midsilk (silks had emerged from 50% of the plants in a plot) differed among hybrids (Table 2). The hybrids produced by crossing the three lines selected for resistance to aflatoxin accumulation (Mp313E, Mp715, and Mp717) were among the latest to flower. These hybrids also exhibited the lowest levels of aflatoxin accumulation and low levels of ear rot and *A. flavus* biomass. The correlations between days to midsilk and ear rot, aflatoxin concentration, and fungal biomass were significant and negative: $r = -0.44$, $P = 0.020$; $r = -0.64$, $P = 0.0002$; and $r = -0.44$, $P = 0.020$, respectively. Because the parental inbred lines selected for resistance are later maturing than the more susceptible lines included in this investigation, the significant negative correlations were not unexpected. In an investigation conducted in Texas, Betrán and Isakeit (2004) also found that silking date and aflatoxin accumulation were negatively correlated.

In this investigation, the fact that timing of inoculation was related to maturity could account, at least in part, for the significant correlations between days to midsilk and the other traits. Hybrids inoculated on the same day would have been exposed to similar environmental conditions after inoculation. It is not possible to determine for this diallel cross whether maturity is an important component of resistance to aflatoxin accumulation and fungal infection. Delaying planting of earlier maturing hybrids so that all hybrids reach midsilk at the same time would allow for inoculation of all plots on the same day and could reduce potential effects of environmental variation after inoculation. Delayed planting of some plots could, however, introduce additional early season environmental variation related to planting date.

Estimates of GCA effects indicate that both T173 and SC212m, when used in hybrids, contributed to higher levels of ear rot, aflatoxin, and *A. flavus* in the grain (Table 3). Estimates of GCA effects for Mp313E and Mp717 indicate these lines in hybrid combinations contribute to lower ear rot, less aflatoxin, and less *A. flavus* in the grain. Mp715 also contributes to lower levels of *A. flavus* and aflatoxin in the grain. These results are consistent with the results of an earlier investigation (Williams et al., 2008). The results indicate that selecting for either reduced levels of aflatoxin or *A. flavus* biomass would be effective in improving the other trait; however, the estimates of GCA effects for GA209 contradict this. The GCA effect for ear rot for GA209 was highly significant and negative (−4.5), and the GCA effect for *A. flavus* biomass was also significant and negative (−0.4). The GCA effect for aflatoxin accumulation was, however, highly significant and positive (0.37). These results indicate that although GA209 could be used successfully to produce maize hybrids with lower levels of ear rot and *A. flavus* infection, aflatoxin would not be correspondingly reduced. When evaluated as an inbred per se, GA209 exhibited relatively low levels of ear rot, but high levels of aflatoxin accumulation (Henry et al., 2009). The performance of GA209 as an inbred and in hybrid combinations was consistent with respect to aflatoxin accumulation and ear rot.

**Table 3**
Estimates of general combining ability (GCA) effects for ear rot, aflatoxin concentration, and *A. flavus* biomass for eight parental lines of a diallel cross grown at Mississippi State in 2008 and 2009.

| Parental line | GCA effect | | |
|---|---|---|---|
| | Ear rot[a] % | Aflatoxin[b] ln(ng/g+1) | *A. flavus* biomass[c] mg/g |
| T173 | 6.9** | 1.42** | 0.6 |
| SC212m | 2.3* | 1.02** | 1.2** |
| CI66 | 2.4* | 0.61** | 0.2 |
| GA209 | −4.5** | 0.37** | −0.4* |
| NC408 | −1.4 | 0.09 | 0.3 |
| Mp313E | − 2.2* | −0.95** | −0.5* |
| MP717 | −2.9** | −1.23** | −0.8** |
| Mp715 | −0.6 | −1.34** | −0.6** |

[a] Ear rot was visually rated as a percentage of rotted kernels on 10 ears per plot.
[b] Aflatoxin concentrations were transformed [ln($y$ + 1), where $y$ = aflatoxin concentration] before statistical analysis.
[c] *A. flavus* biomass was calculated as the actual ratio of *A. flavus* to maize biomass based on extracted genomic DNA from inoculated ears.
* Significantly different from 0 at $P < 0.05$.
** Significantly different from 0 at $P < 0.01$.

Further investigation will be needed to determine whether lower levels of *A. flavus* infection and aflatoxin accumulation in hybrids produced from Mp313E, Mp715, and Mp717 are inextricably linked. If so, the genetic basis of resistance to *A. flavus* infection exhibited by GA209 and its hybrids differs from that of Mp313E, Mp715, and Mp717. Gene mapping and functional genomics investigations should be useful in understanding the genetic basis of resistance to aflatoxin accumulation. The results of this investigation, however, indicate that in breeding maize for resistance to aflatoxin accumulation, selection should be for either reduced aflatoxin or for both reduced *A. flavus* infection and aflatoxin accumulation.

SCA effects associated with aflatoxin accumulation were significant and positive for NC408 × SC212m, SC212m × T173, Mp715 × T173, and Mp313E × SC212m, but significant and negative for GA209 × SC212m, Mp717 × NC408, and Mp313E × Mp715 (Table 4). The significant negative SCA effect for Mp313E × MP715 is especially interesting because both parental lines also exhibited significant negative GCA effects for aflatoxin accumulation (Table 3). This indicates that the genes for resistance to aflatoxin accumulation likely differ in Mp313E and Mp715. This is consistent with results of other investigations that indicated that quantitative trait loci (QTL) associated with resistance to aflatoxin accumulation occurred on different chromosomes of Mp313E and Mp715. QTL on chromosomes 1, 3, 5, and 10 of Mp715 and chromosomes 2 and 4 of Mp313E had significant phenotypic effects on aflatoxin accumulation (Brooks et al., 2005; Warburton et al., 2010). Although SCA was a significant source of variation in the inheritance of resistance to *A. flavus* infection, SCA effects associated with *A. flavus* biomass were significant for only two hybrids. SCA was significant and positive for NC408 × SC212m, the hybrid that exhibited the highest levels of both aflatoxin accumulation (1303 ng/g) and *A. flavus* biomass (7.3 ng/g). The SCA effect associated with *A. flavus* biomass was significant and negative for Mp715 × SC212m.

**Table 4**
Estimates of specific combining ability (SCA) effects for ear rot, aflatoxin concentration, and *A. flavus* biomass in 28 $F_1$ hybrids constituting a diallel cross grown at Mississippi State in 2008 and 2009.

| Hybrid | Ear rot[a] % | Aflatoxin[b] ln(ng/g + 1) | *A. flavus* biomass[c] mg/g |
|---|---|---|---|
| NC408 × SC212m | 2.7 | 1.10** | 3.0** |
| CI66 × T173 | −1.9 | −2.4 | 0.4 |
| GA209 × T173 | −3.8 | −0.29 | 0.1 |
| NC408 × T173 | −6.9** | −0.13 | −0.9 |
| SC212m × T173 | −0.6 | −1.12** | 0.0 |
| CI66 × SC212m | 6.5** | −0.43 | −0.1 |
| CI66 × GA209 | −1.7 | 0.19 | −0.1 |
| Mp715 × T173 | 11.0** | 0.94 | 0.2 |
| Mp313E × T173 | 2.6 | 0.47 | 0.2 |
| GA209 × NC408 | 0.8 | 0.24 | −0.3 |
| Mp313E × SC212m | 2.3 | 0.60 | −0.7 |
| GA209 × SC212m | −2.9 | −0.78 | −0.4 |
| Mp717 × T173 | −0.4 | 0.37 | 0.0 |
| CI66 × NC408 | −2.4 | −0.31 | −0.7 |
| Mp717 × SC212m | −3.7 | 0.45 | −0.8 |
| Mp715 × SC212m | −4.4 | 0.17 | −1.0* |
| GA209 × Mp313E | 0.4 | 0.34 | 0.1 |
| CI66 × Mp717 | 0.4 | 0.37 | −0.1 |
| CI66 × Mp313E | −0.2 | 0.05 | 0.3 |
| CI66 × Mp715 | −0.7 | 0.37 | 0.3 |
| GA209 × Mp715 | 2.4 | 0.41 | 0.3 |
| Mp313E × NC408 | 4.7 | 0.10 | −0.4 |
| GA209 × Mp717 | 4.8 | −0.11 | −0.3 |
| Mp715 × NC408 | −0.7 | −0.41 | −0.5 |
| Mp717 × NC408 | 1.7 | −0.59 | −0.2 |
| Mp313E × Mp717 | −2.5 | −0.30 | −0.3 |
| Mp715 × Mp717 | −0.4 | −0.20 | −0.6 |
| Mp313E × Mp715 | −7.3** | −1.27** | 0.1 |

[a] Ear rot was visually rated as a percentage of rotted kernels on 10 ears per plot.
[b] Aflatoxin concentrations were transformed [ln(*y* + 1), where *y* = aflatoxin concentration] before statistical analysis.
[c] *A. flavus* biomass was calculated as the actual ratio of *A. flavus* to maize biomass based on extracted genomic DNA from inoculated ears.
* Significantly different from 0 at $P < 0.05$.
** Significantly different from 0 at $P < 0.01$.

## 4. Conclusion

Both GCA and SCA were significant sources of variation for ear rot, aflatoxin accumulation, and *A. flavus* biomass; however, the relative contribution of GCA was greater than that of SCA for all traits. For aflatoxin accumulation, both GCA × years and SCA × years were highly significant; however, neither was significant for *A. flavus* biomass. Lack of repeatability of results would be expected to be a greater problem with selection for resistance to aflatoxin accumulation than resistance to *A. flavus* infection as indicated by fungal biomass. The estimates for GCA effects for Mp313E, Mp715, and Mp717 indicate that these germplasm lines should be useful in breeding for resistance to both *A. flavus* and aflatoxin accumulation. Results from an earlier investigation indicated that Mp715 and Mp717 could also be useful sources of resistance to accumulation of fumonisin (Williams and Windham, 2009). The highly significant correlation between *A. flavus* infection and aflatoxin accumulation would appear to indicate that selecting for a reduction in one trait would reduce the other when these sources of resistance are utilized in a breeding program. The relative contribution of GCA and SCA indicates that breeding strategies that maximize GCA should be most effective. The significance of GCA × years and SCA × years for aflatoxin accumulation and the lack of significance for *A. flavus* biomass indicate that greater progress might be made in reducing fungal biomass than aflatoxin accumulation. Although the negative estimates of GCA effects for Mp313E, Mp715, and Mp717 provide evidence of an association between aflatoxin accumulation and *A. flavus* infection, the relative low levels of fungal infection and high levels of aflatoxin accumulation in the GA209 hybrids contradict this. Further study of additional germplasm should be undertaken to determine whether resistance to fungal infection and aflatoxin accumulation are associated with the same or different genes. Such information would be useful in designing efficient breeding programs.

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